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# In Vitro Study of Antibacterial Activity of Many Topical Creams Marketed In Gaza Strip

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***Abstract.*** *Bacterial Superficial skin infections are often treated with topical antibiotic preparations applied directly to the skin which offer a useful alternative to oral and parenteral agents in some conditions and have many advantages such as easy to use, lower side effects, higher drug concentrations in the site of infection, lower risk of developing bacterial resistance and more economical than other routes. Recently, many strategies have been proposed to conflict bacterial resistance. Combination therapy and using novel drug delivery systems are the main approaches in this field. A large number of antibacterial topical skin preparations currently available in Gaza markets which manufactured by different pharmaceutical companies. Some of these preparations are pharmaceutical alternatives which contain the same therapeutic moiety but differ in salt or ester form, in the dosage form or in the strength. In this study we evaluated the antibacterial activity of four types of antibiotic creams used widely in Gaza dermatological clinics, fusidic acid, silver sulfadiazine, gentamicin and clindamycin. For this purpose, in vitro disk diffusion method, which is one of the most widely used antimicrobial susceptibility testing methods in routine clinical laboratories, was used to examine antibacterial activity of these topical preparations as described by EUCAST. Furthermore, we prepared a combination of different used antibiotic creams and examined the antibacterial activity of these combinations.*

**Keywords**: Fusidic acid; Silver sulfadiazine; Gentamicin; Clindamycin; Antibacterial activity; in vitro disk diffusion method.

## INTRODUCTION

Skin and soft tissue infections are a common problem encountered in clinical practice which range from superficial epidermis infection to life-threatening necrotizing fasciitis (Templer and Brito, 2009).The majority of these infections are caused by bacteria and are referred to as acute bacterial skin and skin structure infections. Some cases are caused by viruses and fungi (Chahine and Sucher, 2015, Ki and Rotstein, 2008). Bacterial Superficial skin infections are often treated with topical antibiotic preparations applied directly to the skin which offer a useful alternative to oral and parenteral agents in some conditions and have many advantages such as easy to use, lower side effects, higher drug concentrations in the site of infection, lower risk of developing bacterial resistance and more economical than other routes (Cesur, 2002). Numerous topical antibacterial products are available over-the-counter (OTC) or by prescription and may contain different active anti-infective agents which present in various pharmaceutical dosage form (ointment or cream). However, the prevalent use of commonly available topical antibiotics (particularly mupirocin and fusidic acid) has promoted bacterial resistance in some settings, limiting the therapeutic efficacy of such agents (Chen et al., 2010). Therefore, Given global concerns regarding antibiotic resistance especially for some species, such as S. aureus, the appropriate use of topical agents and the prevention of further resistance are critical. Recently, many strategies have been proposed to conflict bacterial resistance. Combination therapy and using novel drug delivery systems are the main approaches in this field (Uchil et al., 2014).

A large number of antibacterial topical skin preparations currently available in Gaza markets which manufactured by different pharmaceutical companies. Some of these preparations are pharmaceutical alternatives which contain the same therapeutic moiety but differ in salt or ester form, in the dosage form or in the strength. In this study we evaluated the antibacterial activity of four types of antibiotic creams used widely in Gaza dermatological clinics, fusidic acid, silver sulfadiazine, gentamicin and clindamycin. We used three brands for each type of cream except two brands for clindamycin to compare between the therapeutic effect of different brands. One of these brand manufacture by local companies in Gaza and West Bank and others by international companies. For this purpose, in vitro disk diffusion method, which is one of the most widely used antimicrobial susceptibility testing methods in routine clinical laboratories, was used to examine antibacterial activity of these topical preparations as described by EUCAST. Furthermore, we prepared a combination of different used antibiotic creams and examined the antibacterial activity of these combinations.

## INSTRUMENTS AND MATERIALS

### Instruments

Balance, Hot plate, Autoclave, Incubator

### Materials

**Topical skin creams**

The following skin pharmaceuticals from different manufacturer were tested. All formulations were creams:
**Fucidic acid 2%:** FM, FL, FJ .

**Silver sulfadiazine 1%:** SM, SA**,** SB.

**Gentamicin 0.1%:** GM, GZ, GD

**Clindamycin 2%:** CD, CM**.**

**Combination :** different antibiotic creams from the same company (M company) were combined by mixing the same amount of each cream to produce a homogenous formula as the following;

FM & SM creams; FM & GM creams; FM & CM creams; CM & GM creams; FM, GM& SM creams.

**Culture media**
Blood agar from HiMedia Laboratories Pvt.Ltd.
McConkey agar from HiMedia Laboratories Pvt.Ltd.
Muller Hinton agar from HiMedia Laboratories Pvt.Ltd.

**Bacteria species**

Four types of bacteria strains were used: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *kliebsella spp*, *Pseudomonas aeruginosa*. These bacterial species were obtained in cooperation with the Plasma Advanced Medical Laboratory in Gaza City.

**Methods**

**Study design**

An in-vitro comparative study of the antibacterial properties of currently available antibacterial topical formulations prescribed by the dermatological clinic of Gaza Strip. We have adopted an unconventional approach whereby we test the formulations in their marketed form and not merely the designated active substance of each formulation. For this purpose, a Disc Diffusion Method for Antimicrobial Susceptibility Testing was selected.

**Preparation of bacterial culture:**

Blood agar and McConkey agar preparation according to the manufacturing company.
Mueller-Hinton agar is considered the best medium to use for routine susceptibility testing of nonfastidious bacteria for the following reasons:
It shows acceptable batch-to-batch reproducibility for susceptibility testing · It is low in sulfonamide, trimethoprim, and tetracycline inhibitors · It supports satisfactory growth of most nonfastidious pathogens · A large body of data and experience has been collected concerning susceptibility tests performed with this medium (Winn, Jr., W., et al. 2006)

**Preparation of antibiotic disk**

Disks of antibiotics were prepared by immerse paper disc, with 5.0 mm in diameter, Equal amounts of antibiotic preparations were applied for a definite period which allow the disc to be saturated with cream. Kirby-Bauer disk diffusion method was used to tests the effectiveness of antibiotics on a specific microorganism. An agar plate is first spread with bacteria, then antibiotic disks are added. Each petri dish contains a pure type of bacteria and type of antibiotic discs of the same active ingredient having a different generic name as in figure 1. The label were added in the bottom of the plates to facilitate distinguish between different antibiotic discs.


**Figure 1:** Disc Diffusion Method for Antimicrobial Susceptibility Testing

**Kirby-Bauer disk diffusion susceptibility**

The Kirby-Bauer disk diffusion susceptibility test protocol has been implemented as described in the American Society for Microbiology © 2016(Jorgensen, J. H., and J. D. Turnidge. 2007.)

**Antibacterial Susceptibility Testing**

Measure the inhibition zone, which is a circular clear area around the antibiotic disks where the bacterial colonies do not grow, as measured by a standard ruler in mm to investigate the effect of different antibiotic-containing disks (edge should be taken as the point of inhibition as judged by the naked eye) (**Figure 2**). the larger zone of inhibition around an antibiotic-containing disk indicates that the antibiotic cream in the disk is more effective against bacteria than others.

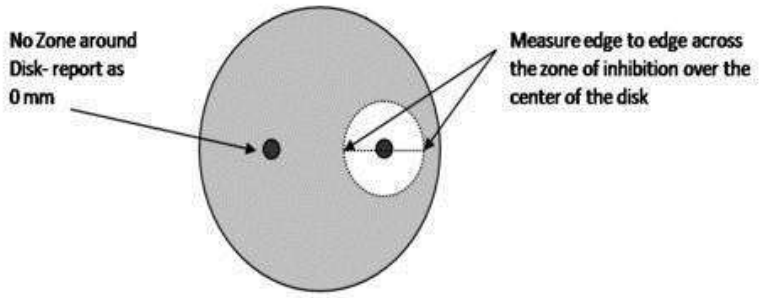


**Figure 2:** Measuring the inhibition zone

**RESULTS**

**Fusidic acid 2%**

The antimicrobial effect of fusidic acid 2% cream was seen against Gram-positive bacteria (*streptococcus pyogenes* and *staphylococcus aureus*). However fusidic acid was observed to be more effective against *staphylococcus aureus* than *streptococcus pyogene* demonstrated by larger inhibition zone. No effect was observed against Gram-negative bacteria (*Pseudomonas aeruginosa*, *E coli* and *kliebsella*). In comparison between different brands, all creams have nearly the same effect on bacteria with slightly higher inhibition zone was recorded for FL, FJ creams. (**Table 1**).

**Silver sulfadiazine1%**

The antibacterial effect of silver sulfadiazine cream was seen only against pseudomonas aeruginosa. No effect was observed against, *E coli, streptococcus pyogenes, staphylococcus aureus* and *klebsiella*. In comparison between different brands, SA has the highest inhibition zone which is 1.5 fold greater than SM, SB creams.( **Table 1**).

**Gentamicin 0.1%**

The antimicrobial effect of gentamicin cream was seen against all bacteria species used in this study. However, gentamicin was observed to be most effective against *E. coli* than other types of bacteria demonstrated by larger inhibition zone and least effective against *staphylococcus aureus* demonstrated by smaller inhibition zone. In comparison between different brands, all creams from the different manufacturers have nearly the same effect on each type of bacteria as they give the same inhibition zone. **(Table 1**).

**Clindamycin 2%**

The antimicrobial activity of clindamycin cream was seen against *staphylococcus aureus, streptococcus pyogenes*, and *Kliebsella*. No effect was observed against *pseudomonas aeruginosa* and *E coli* .In comparison between different brands, CD showed higher effect than CM against both types of bacteria.(**Table 1**).

**Combination (synergism)**.

The antimicrobial activity of compound antibiotic creams applied from the same source, M company, the antibacterial activity of synergism of antibiotic creams gives greater activity than using these creams each of alone for some types of bacteria, while others are not affected by this synergy as is evident in **(Table 2)**.

**Table 1:** Inhibition zone diameterof antibiotic creams in disk diffusion method.

| | | | Inhibition zone diameter (mm) | | | | |
|---|---|---|---|---|---|---|---|
| Symbol | Active ingredient | Company | Staphylococcus aureus | Pseudomonas aeruginosa | E. Coli | Klebsiella | streptococcus pyogenes |
| FM | Fusidic acid | M | 23 | 0 | 0 | 0 | 18 |
| FL | Fusidic acid | L | 26 | 0 | 0 | 0 | 21 |
| FJ | Fusidic acid | J | 25 | 0 | 0 | 0 | 21 |
| | | | | | | | |
| SM | Silver sulfadiazine | M | 0 | 9 | 0 | 0 | 0 |
| SA | Silver sulfadiazine | A | 0 | 14 | 0 | 0 | 0 |
| SB | Silver sulfadiazine | B | 0 | 10 | 0 | 0 | 0 |
| | | | | | | | |
| GM | Gentamicin | M | 13 | 15 | 21 | 19 | 20 |
| GB | Gentamicin | Z | 13 | 17 | 24 | 20 | 20 |
| GD | Gentamicin | D | 13 | 16 | 24 | 20 | 21 |
| | | | | | | | |
| CD | Clindamycin | D | 16 | 0 | 0 | 18 | 18 |
| CM | Clindamycin | M | 12 | 0 | 0 | 15 | 8 |

**Table 2:** Inhibition zone diameter of combined antibiotic creams from Megapharm company in disk diffusion method.

| | | Inhibition zone diameter (mm) | | | |
|---|---|---|---|---|---|
| Symbol | Combination | Staphylococcus aureus | E. Coli | Klebsiella | streptococcus pyogenes |
| FS | Fusidic acid, Silver sulfadiazine (FM & SM creams) | 28 | 0 | 0 | 13 |
| FG | Fusidic acid, Gentamicin (FM & GM creams) | 26 | 28 | 19 | 25 |
| CF | Fusidic acid, Clindamycin (FM & CM creams) | 26 | 0 | 17 | 19 |
| CG | Clindamycin, Gentamicin (CM & GM creams) | 22 | 22 | 20 | 24 |
| Tri | Fusidic acid, Silver sulfadiazine, Gentamicin (FM, GM& SM creams) | 27 | 28 | 16 | 23 |

## DISCUSSION

We have investigated the in vitro activity of several widely marketed topical antibiotic skin creams marketed in the Gaza Strip. For this study we use a Disc Diffusion Method which is described by the American Society for Microbiology and European Committee on Antimicrobial Susceptibility Testing EUCAST. Disk diffusion is one of the oldest approaches to antimicrobial susceptibility testing and remains one of the most widely used antimicrobial susceptibility testing methods in routine clinical laboratories (EUCAST, 2020). Many studies used this method to investigate the antibacterial activity of topical preparations (Sedef Gocmen et al., 2008). Mueller-Hinton agar was used as media for antimicrobial susceptibility testing because it is a non-selective and non-differential medium which suitable for the growth of almost all organisms. Furthermore, it contains starch that absorbs toxins released from bacteria, so that they cannot interfere with the antibiotics. It is a loose agar which allows for better diffusion of the antibiotics than most other plates. Better diffusion leads to a truer zone of inhibition.

The results showed that all the antibiotics used in the study had an almost equal effect on the types of bacteria used in this study, except for Silver sulfadiazine from A company , which gave an increase in the effect of up to 4 mm on *Pseudomonas aeruginosa*, and Clindamycin from D company gave a stronger increase in the effect Against *streptococcus pyogenes* up to 10 mm, it also gave an increase in the effect of up to 4 mm against *Staphylococcus aureus*, as shown in **Table 1**.

Also, the results showed that fusidic acid alone has the highest antibacterial activity against *Staphylococcus aureus* as shown in **Table 1**. However, the synergism of fusidic acid from M company with gentamicin containing cream from the same company slightly increase of inhibition zone diameter ( IZD) had been observed ( 23mm for fucidic acid alone and 26mm for combination). Also, when a triple combination of fusidic acid, gentamicin and silver sulfadiazine was prepared, IZD increased to 27mm., this indicates that these combinations increase the antibacterial activity of fusidic acid with the dominant effect observed for fusidic acid. Furthermore, clindamycin and gentamicin have a similar effect against *staphylococcus aureus* (IZD 12mm and 13mm, respectively). However, the combination of these antibiotic creams shows a higher IZD (22mm). with this in mind, a combined clindamycin and gentamicin preparation can be a valuable option to get a higher effect against *staphylococcus aureus*. Regarding *streptococcus pyogenes*, fusidic acid, gentamicin ,and clindamycin exhibited effect against *streptococcus pyogenes* but the higher antibacterial activity was observed for gentamicin (**Table1**). From results shown in **Table 2**, the combination of gentamicin with fusidic acid, clindamycin with fusidic acid or fusidic acid and silver sulfadiazine with gentamicin as a triple combination, antibacterial activity increased against *streptococcus pyogenes*. But to be in consideration that triple combination doesn't have greater antibacterial effects.

So, the dual combination is encouraged because it will boost activity and will be cost-effective. Regarding *E. coli*, gentamicin is the only antibiotic cream that has shown antibacterial activity against *E. coli*. On the other hand, the double combination of gentamicin with fusidic acid or clindamycin and triple combination with fusidic acid and silver sulfadiazine leads to increased IZD which indicates higher antibacterial activity while the combination of fusidic acid and silver sulfadiazine did not give any significant effect as well as the combination of fusidic acid. Clindamycin has did not affect to *Klebicella*, gentamicin and clindamycin are the only antibiotic creams that have shown antibacterial activity against *Klebsiella*. A double mixture of gentamicin, fusidic acid, or gentamicin and clindamycin showed the same activity against *Klebsiella*, as in the case of gentamicin and clindamycin use alone, there was no effect of combining fusidic acid and silver sulfadiazine against *Klebsiella*. By contrast, reduce the antibacterial activity of this antibiotic when using a triple group. It can be speculated that the reduced activity is due to silver sulfadiazine which may interfere with the activity of the antibiotics of gentamicin and clindamycin because in a double mixture of gentamicin with fusidic acid the same activity is observed as in gentamicin alone.

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